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Enhanced Interferon Response to Murine Leukemia Virus by Ascorbic Acid

BENJAMIN V. SIEGEL

Department of Pathology, University of Oregon Medical School, Portland, Oregon 97201

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BALB/cJ mice, on an ad libitum regimen of 250 mg% L-ascorbate in their drinking water, displayed augmented levels of circulating interferon after stimulation with murine leukemia virus.

The mechanism of action of ascorbic acid in its suggested protection against some viral infections, such as the common cold in humans (7), is not understood. The possibility of its role in viral inactivation (6), as immunologic enhancer (1), and in interferon stimulation (7) has been variously postulated. With regard to the latter, induced or exogenously administered interferon has been reported to bring about transient remissions in patients with acute leukemia (2, 11), and has been demonstrated to be experimentally efficacious in altering leukemia of viral etiology in mice (5). We describe here the enhancing effect of a vitamin C regimen on interferon induction by a murine leukemogenic virus.

BALB/cJ male mice were fed L-ascorbate (250 mg%) in their drinking water ad libitum, beginning at 24 days of age, for 3 months; a similar set of control animals remained on untreated water. At this time mice were stimulated to interferon production with Rauscher leukemia virus (RLV). RLV represented the 10th BALB/c spleen passage in this laboratory from a stock obtained from F. J. Rauscher of the National Cancer Institute. Mice were inoculated intraperitoneally with 0.20 ml of a supernatant of 10% RLV-infected spleen homogenate clarified twice at 2,500 rpm for a total of 40 min at 4 C in a PR-2 International refrigerated centrifuge. Twenty-seven hours later animals were bled for interferon assay (9) by a colorimetric method for quantifying cytopathic effects in mouse L-cell monolayers (3) with vesicular stomatitis virus (VSV) as the challenge virus. Individual blood samples were collected by orbital sinus puncture and allowed to clot, and the serum was removed. Serum pools were prepared from two mice per experimental group and, before assay, were exposed for 5 min at 25 cm to a 15-W General Electric germicidal ultraviolet lamp (10).

Volumes (1 ml) of serial dilutions of sera were used for assay, and interferon titers were expressed as the reciprocal of the dilution depressing dye uptake inhibition by VSV by 50%. Assays were carried out twice with two different dosages of challenge virus, namely, 16×10^4 and 8×10^4 plaque-forming units (PFU) of VSV. A standard reference interferon preparation (National Institutes of Health Mouse Reference Interferon G002-904-511) was included with each assay. The viral inhibitor was partially labile at pH 2.0, not sedimentable at 100,000 $\times$ *g*, nondialyzable, inactivated by trypsin, nontoxic to L cells, and did not directly inactivate VSV.

BALB/cJ mice on the ascorbate regimen showed on an average (Table 1) a 62% increase in circulating interferon level when the assay was performed with a virus dose challenge of 16×10^4 PFU and a 145% increase at the lower dose virus challenge of 8×10^4 PFU, or an average increase of 104% based on the two assays. Since the primary host defense in virus disease is probably the production of interferon, the enhanced interferon response noted here in mice on the ascorbate regimen might suggest a mechanism to account, at least in part, for instances where vitamin C may be proven to provide protective effects against viral infection. Although blood ascorbate levels were not determined, Schlegel and co-workers noted (8) that the average concentration of ascorbate in the urine of mice on a similar 250 mg% regimen was 400 mg% compared to 16 mg% in untreated mice and that this was related to high ascorbate levels in the serum. It may be of additional interest that the interferon-stimulating agent was a leukemogenic virus (RLV), and that Gresser and his associates (4) were able to inhibit development of the splenomegalic response in mice to this virus by interferon administration. Further experiments are in

TABLE 1. *Assays of serum interferon carried out in mouse L-cell monolayers at two different dosages of vesicular stomatitis challenge virus*

Mouse group	Treatment	Interferon titers[a]	
		16×10^4 PFU	8×10^4 PFU
1	Ascorbate in water	480 (515)[b]	1,550 (1,668)
2	Ascorbate in water	550	1,785
3	Control	295 (318)	590 (682)
4	Control	340	775
	NIH reference	4,710	16,400

[a] Titers are expressed as the reciprocal of the dilution depressing dye uptake inhibition by VSV by 50% (DDU50-VSV/ml).

[b] Figures in parentheses are the average interferon titers for the two groups in a given treatment.

progress to establish whether ascorbate may also play a role in influencing the immune response.

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